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TECHNICAL MANUSCRIPT 397

LATE-DEVELOPING ORANGE PUSTULE OF PUCCINIA GRAMINIS VAR. TRITICI, RACE 56: POSSIBLE CONNECTION WITH CERTAIN MUTANTS AND AMPHISPORES

Anthony V. Mattie
William E. Miller
Robert G. Emge

APRIL 1967

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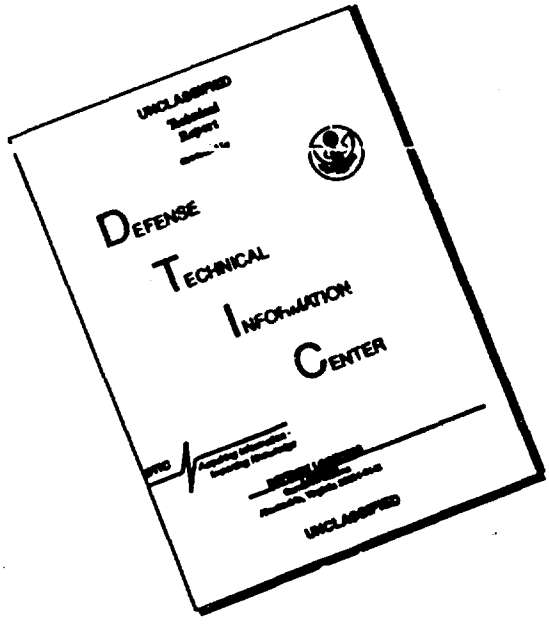

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LATE-DEVELOPING ORANGE PUSTULE OF *PUCCINIA GRAMINIS* VAR. *TRITICI*, RACE 56: POSSIBLE CONNECTION WITH CERTAIN MUTANTS AND AMPHISPORES

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BIOLOGICAL SCIENCES LABORATORY

Project 1C522301A061

April 1967

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ABSTRACT

Flecks appeared in leaves of Baart wheat (C.I. 1697) 13 days after inoculation with uredospores of an orange isolate from normal race 56 of Puccinia graminis Pers. var. tritici (Eriks. & E. Henn.) Guyot. The resulting pustules were orange; most remained orange, but some reverted and produced spores adjoining the orange pustules that were the parental red-brown color. Spores from the orange pustules differed from those of normal race 56 in shape, appearance of cytoplasm, cell wall, and stainability. Although germination of spores on agar from the orange pustules was low (4 to 5%) and that from the reverted portion of the pustules was high (90 to 95%), both types of spores were highly infective on Baart wheat. Sporulation resulting from inoculation with uredospores from the orange pustules occurred in 15 to 18 days; with uredospores from the reverted red-brown pustules, in the usual 8 to 10 days. Orange and reverted pustules consistently produced typical one- and two-celled teliospores. Reactions on differential varieties inoculated with spores from orange pustules were different from those of race 56 (most varieties were resistant); however, reactions on the differential varieties inoculated with spores from the red-brown reverted pustules were similar to those of race 56.

These findings closely parallel those of other investigators on the behavior and appearance of amphispores (formerly called mesospores) in other Puccinia species and of certain orange mutants, aberrants, and dissociants in P. graminis.

I. INTRODUCTION

Variability in shape, size, color, and behavior of both uredia and uredospores in Puccinia graminis Pers. var. tritici (Eriks. & E. Henn.) Guyot and in other Uredinales is well known. However, a spore form, intermediate in some respects between uredospore and teliospore, has not been reported for P. graminis, although such spores, called mesospores by Dietel[1] and later amphispores by Carleton,[2] have been reported for some other species of Uredinales.[3-7]

Arthur[3] states, "In some species there occur specially developed urediniospores with thicker and sometimes more highly colored walls, which act as resting spores and are known as amphispores." He also states that although the specially developed uredospores clearly belong to the repeating stage, they may differ from uredospores of the same species in color of contents, shape, markings, thickness of wall, pore arrangements, and in the persistence of pedicels, yet they give rise to a mycelium that bears uredospores characteristic of the species.

Carleton[5] states that these amphispores must be considered distinct because of the presence of uredospores and teliospores and the morphological differences among all three. He described an inoculation with uredospores of P. vexans as "rust spots" appearing in 12 days, followed in 9 more days with one sorus of amphispores. Carleton quotes W. G. Farlow as saying that he had never observed germination of the unicellular spores, so he saw no reason why they might not be uredospores. On the other hand, however, he felt that their general appearance and the density of the spore wall would lead one to suppose they were teliosporic.

Hardison[8] reported that amphispores found on Poa pratensis L., bluegrass, infected with Puccinia poae-sudeticae (West) Jorstad (a species in which telia are rarely produced) had generally persistent, colorless pedicels one to two times the length of the spore, minutely echinulate spore walls, and six scattered pores. He also states that they would not germinate at maturity. However, a germination of 2% was obtained when they were weathered for 3 months and a 15 to 20% germination was obtained 2 months later. Plants of P. pratensis inoculated with amphispores produced uredia 7 days after inoculation, but amphispores had not developed 26 days after inoculation. "Urediospores which accompanied the amphisporic inoculum were non-viable. The uredia which developed from the inoculation with amphispores were characteristic in every respect for the species. The abundant capitate paraphyses accompanying the uredospores provide unmistakable evidence of the connection of the amphispores in the life history of P. poae-sudeticae."[8]

In this paper we are reporting on the behavior of the progeny of orange spores that developed on wheat leaves inoculated with spores of P. graminis var. tritici, race 56. We suggest that the orange spores are amphispores and that some orange pustules termed "mutants," "aberrants," and "dissociants" by investigators may, in fact, be pustules containing amphispores. As far as we are aware, no such spore form has been described for P. graminis var. tritici.

Although Cummins[6] describes amphispores occurring in P. vexans Farl., P. atrofusca (Dudl. & C. H. Thompson) Holw., and other Puccinia species, he uses Persoon's interpretation of Puccinia in which amphispores are not mentioned. In a later paper[7] he states that amphispores occur in P. angusii, sp. n.

The orange pustules and their spores discussed here were first found and isolated by the senior author in 1962 from greenhouse-grown Baart wheat plants that had been inoculated with P. graminis var. tritici, race 56. Flecks did not appear in the usual 6 to 7 days on the wheat plants inoculated with this isolate, so the plants were discarded.

A similar orange pustule was found again in 1963 under similar circumstances and treatments. However, this pustule was found among red-brown pustules that were 3 to 4 weeks old. It was assumed that this was a late-developing pustule because it was small compared with the normal pustules from race 56. Baart wheat plants were inoculated with an isolate from this pustule, given an overnight dew period at 70 F and 100% relative humidity, and incubated at 70 F and 65% relative humidity. Again, there was no flecking in 6 to 7 days, but the plants were observed daily. Flecks were visible on the 13th day after inoculation and sporulation was observed on the 15th day. The color and shape of the pustule did not change through the 20th day when the plants were discarded because of mildew. Spores from this pustule were not used because of the contaminating mildew.

Additional orange pustules were observed from time to time, but isolations were not made until September 1965, at which time the senior author initiated a series of investigations.

II. MATERIALS AND METHODS

Soil-filled clay pots (4-inch diameter) were seeded (25 to 30 seeds per pot) with Baart wheat C.I. 1697. When the plants were 6 to 8 inches tall, secondary leaves were removed and kept trimmed during each run, leaving primary leaves only. The potted plants were divided into three groups and inoculated with uredospores from orange pustules of race 56, from red-brown pustules of race 56, and from reverted* portions of orange pustules. The uredospores were suspended in Freon-113[9] and atomized onto

* A term selected to describe a single pustule that produces two types of spores, one section containing orange spores and the adjoining section containing red-brown spores (Fig. 1).

the plants. The plants were placed in a dew chamber, exposed to an overnight dew period at 70 F and 100% relative humidity, transferred to a greenhouse, held at 70 F and 65% relative humidity, and observed daily.

Flecks appearing before the 12th day after inoculation with spores from the orange pustule were removed. Flecks appearing on plants inoculated with spores from red-brown pustules of race 56 and with spores from the reverted portion of orange pustules were allowed to develop. Spores from each treatment were collected[10] and stored separately.

Infection types produced by each of the three inocula were observed on standard stem rust of wheat differentials[11] (Table 1). The differential varieties were grown and handled as was Baart.

TABLE 1. REACTION OF DIFFERENTIAL WHEAT VARIETIES INOCULATED WITH THREE KINDS OF UREDOSPORES

	Mean Infection Types[a]		
Wheat Variety	Inoculum from Red-brown Pustule of Normal Race 56	Inoculum from Orange Pustule[b]	Inoculum from Reverted Portion of Orange Pustule[c]
Little Club	4	4	4
Marquis	4	4	3,4
Reliance	4	4	4
Kota	3,4	3	3,4
Arnautka	1	0;	0; 1[d]
Mindum	1	0;	1[d]
Spelmar	1	0;	0; 1[d]
Kubanka	3,4	1,2	3,4[d]
Acme	3,4	0;[d]	1[d]
Einkorn	1	0; 1	1,2
Vernal	1	0;	0; 1
Khapli	1	0;	0; 1
Lee	1	0; 1	1
Selkirk	1	0;	1

a. 0; = very resistant; 1 = resistant; 2 = moderately resistant; 3 = moderately susceptible; and 4 = very susceptible.[11]
b. Orange pustule, flecks in 13 to 15 days, sporulation in 15 to 18 days.
c. Reverted portion of orange pustule, flecks in 6 to 7 days, sporulation in 8 to 10 days.
d. Necrotic.

Fresh, unhydrated spores from orange pustules, red-brown pustules of race 56, and reverted portions of orange pustules were seeded on 5-ml portions of a 1% distilled water agar in plastic petri dishes in a settling tower.[12] Three replications from each group were incubated in the dark for 17 hours at 70 F, during which time they were covered with damp toweling. One hundred spores were counted on each plate. Germination tests were made with spores from each of 9 successive uredial generations.

Orange pustules were counted before any reversion took place and recounted daily. The number that reverted and those that did not change were recorded throughout the lifetime of the plants on three different sets of inoculations.

III. RESULTS

Flecks appeared 13 days after plants were inoculated with spores from the orange pustule; sporulation occurred in 15 to 18 days. Additional flecking occurred as late as 20 days after inoculation. The resulting pustules were orange. Twenty-two days after inoculation, about 20 to 30% of the orange pustules began to produce spores in areas that reverted to the typical red-brown color characteristic of P. graminis var. tritici, race 56.

The orange pustules were round, small, and resembled raised blisters. They gradually increased in diameter and matured in approximately 20 days. Many had a depression in the center. Some of the pustules remained round and, regardless of the number on a leaf, did not coalesce and did not produce secondary rings; others became oval (Fig. 2).

Under the same conditions, plants that were inoculated with uredospores from the red-brown reverted portion of the orange pustules reacted similarly to those inoculated with normal race 56. Flecks appeared in 6 to 7 days; sporulation occurred in 8 to 10 days. Daughter pustules resulting from the inoculations made with uredospores from reverted pustules were the shape and color of normal stem rust pustules. However, some orange segments occasionally were seen in the secondary and tertiary rings.

Spores from the red-brown reverted portion of orange pustules were the same shape as spores from normal red-brown pustules. They also stained similarly. The spores from orange pustules varied in shape. They differed from spores from normal red-brown pustules in shape, thickness of wall, echinulation, stainability, and appearance of cytoplasm (Fig. 3 and 4).

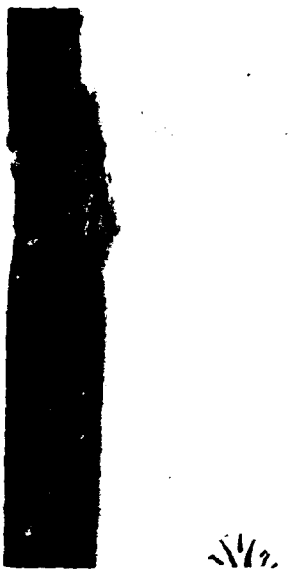

Figure 1. A Reverted Orange Pustule. The lower section is orange, the upper section adjoining it is red-brown.

Figure 2. Orange Pustules on Baart Wheat Plants Inoculated with Spores from Orange Pustules. Note reverted pustule on center leaf.

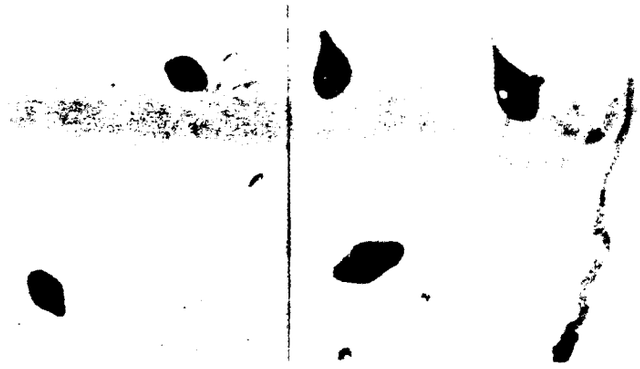

Figure 3. Spores from Orange Pustule. Note variation in shapes. 450X.

Figure 4. Spores from Orange Pustule Showing Thickness of Cell Wall and Appearance of Cytoplasm. 1000X.

On water agar, spores from the orange pustule germinated 4 to 5%, but spores from the reverted portion of the pustule germinated 90 to 95%. Infection of plants was heavy from each inoculation.

Results similar to those above were obtained with spores from each of nine successive uredial generations.

Through these nine generations, orange pustules and reverted portions of orange pustules consistently contained typical one- and two-celled teliospores.

Sets of standard differential varieties,[11] with the additional varieties Lee and Selkirk, were inoculated with spores from red-brown pustules, from orange pustules, and from reverted portions of orange pustules. All flecks were allowed to develop.

With the orange inoculum, flecks appearing after the 12th day developed into orange pustules on varieties susceptible to race 56, but these pustules were much smaller than and differed in shape from those of normal race 56 and those that reverted (Table 1). Twenty-two days after inoculation, some of the pustules from the orange inoculum began to produce spore masses adjoining the orange portions that were red-brown and typical of race 56. Other pustules remained orange.

IV. DISCUSSION AND CONCLUSIONS

Newton and Johnson reported that an orange and a gray-brown pustule appeared in cultures of P. graminis var. tritici, races 9 and 36 respectively. "The orange mutant appeared as a single pustule in a culture which for six generations of uredinia had produced spores of normal color; the greyish-brown mutant appeared in the first uredinial generation of a culture derived from aecia on Berberis vulgaris. The orange mutant still has the characteristic reaction of physiologic form 9, from which it arose; and, similarly, the greyish-brown mutant remains identical pathogenically with form 36."[13] The gray-brown and orange uredospores differed markedly from the normal red-brown uredospores not only in color but in shape, size, and germinability. Germination of orange and gray-brown uredospores was considerably lower than that for the normal red-brown spores, and there was greater variation among consecutive tests.

Barr et al.[14] reported finding a yellow mutant in a culture of red race 2 of P. recondita f. sp. tritici that had been serially propagated by single-pustule transfer during a period when yellow clones were not being grown.

Our orange pustule from race 56 also was found when orange uredospores were not known to exist in P. graminis var. tritici, race 56.

Nelson[15] reported that an orange biotype occurred during an experiment with a mixture of red-brown race 11 and gray-brown race 121 of P. graminis var. tritici. All isolates of the orange biotype produced red-brown and/or gray-brown dissociants after six uredial generations.

The findings reported here differ from Nelson's in that through nine generations only 20 to 30% of our orange pustules reverted to the typical red-brown color of race 56. Nelson's orange biotype produced red-brown and/or gray-brown dissociants in all isolates by the sixth uredial generation and produced only orange uredia through four additional generations. However, Nelson's orange biotype and our orange pustule behaved similarly in that they both produced pustules of the normal red-brown color, as did amphisporic pustules described by Arthur.[3]

Johnston[16] reported that he inspected a pure-line selection of Mediterranean wheat, Texas No. 3015-63, known to be highly resistant to leaf rust. The upper leaves were practically rust-free, but they exhibited marked flecking. About 2 weeks later numerous small, light-colored uredia were observed. When rust from these pustules (given the culture number 199) was transferred to Kanred C.I. 5146, there was no sign of infection at 7 days but at the end of 10 days a few flecks appeared. Other collections that were cultured at the same time showed flecks at the end of 4 to 6 days and developed uredia in 8 to 10 days. Culture 199 did not develop uredia until the 15th day after inoculation. Johnston showed clearly that not only was the incubation period of culture 199 longer than that for race 9, but also that the time required to reach full maturity was longer; the average interval from inoculation to sporulation was nearly twice as long. He further found that the spores of culture 199 were a much lighter color than were those of race 9 and that the uredia were smaller.

Of more than 200 cultures studied at Manhattan, Kansas, from 1926 through 1929, the only culture with a strikingly long incubation period was culture 199. Johnston states that it is apparent that culture 199 is unique both in length of the incubation period and in spore color as reflected in the uredia. His tests on differentials showed that this culture resembled no other described form. He also states that "no straight type-4 infection had been noted for culture 199." The uredia always were smaller and less abundant. He further states that "it appears that culture 199 is a different physiological form although it somewhat resembles physiological form 10." The long incubation period, light-colored spores, and small uredia of culture 199 would place it in a separate category.

It is possible that if Johnston had carried these pustules of culture 199 longer on the same plants, the pustules might have produced red-brown spores as do our reverted pustules arising from inoculations with spores from orange pustules.

Watson[17,18] studied two new orange races of P. graminis var. tritici, NR-1 and NR-2, the latter assumed to be a mutant of NR-1. NR-1[18] may have come initially from an intervarietal cross between P. graminis Pers. var. secalis (Eriks. & E. Henn.) and P. graminis var. tritici. Watson[17,18] notes that both of these races are unusual in that each is avirulent on Acme wheat and that such races are seldom found in North America. However, races avirulent on Acme occur commonly in crosses between P. graminis var. tritici and P. graminis var. secalis.

The orange spores and uredospores from the reverted portion of the orange pustule described in this report are also avirulent on Acme, although the parent race 56 produces susceptible-type pustules on this variety.

We believe that the spores formed in our orange pustules are amphispores. Perhaps some of the colored "mutants" described by other authors were also pustules in which amphispores were being produced. It has been suggested that amphispores serve as overwintering structures for some species,[3,5,8] and it is quite possible that amphispores of P. graminis var. tritici serve the same purpose.

Hyaline spores, varying in shape, and with persistent pedicels, were obtained from gray-brown areas of old orange pustules. They may represent still another spore stage coexisting with amphispores produced in the orange areas.

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14. KEY WORDS: Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report. Key words must be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context. The assignment of links, rules, and weights is optional.

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